MEDICAL COMPOSITION FOR PREVENTING OR TREATING AMYLOID BETA PEPTIDE RELATED DISEASES OR CONDITIONS

A pharmaceutical composition containing isoacteoside to the acteoside is provided, which is able to inhibit formation, accumulation or aggregation of amyloid β peptides, and is thus useful in preventing or treating amyloid beta peptide-associated diseases or conditions, wherein a weight ratio of the isoacteoside to the acteoside is 4:1 to 1:4.
The present invention relates to a pharmaceutical composition for use in preventing or treating amyloid \(\beta\) peptide associated diseases or conditions, which comprises acteoside and isoacteoside as potent components capable of inhibiting formation, accumulation or aggregation of amyloid beta peptides.

US patent No. 7,087,252 B2 discloses a medicinal preparation containing phenylethanoid glycosides extracted from *Cistanche tubulosa* (Schenk.) Wight, said preparation comprising 25-50 wt% of echinacoside and 5-15 wt% of acteoside, which is useful in treating senile dementia. Isoacteoside and other phenylethanoid glycosides are known also being contained in said medicinal preparation.

The applicant of this application in WO 2011/157059 A1 discloses use of isoacteoside or a pharmaceutically acceptable salt thereof in inhibiting the formation, accumulation or aggregation of amyloid \(\beta\) peptide (A\(\beta\)), and use in the fabrication of a medicament for preventing or treating A\(\beta\)-associated diseases or conditions.


In the present application, the inventors continue the research of WO 2011/157059 A1 and obtain a related inventive accomplishment.

Since A\(\beta\) and its aggregates are likely to cause various diseases or conditions in organisms, one object of the present invention is to provide pharmaceutical composition for inhibiting formation, accumulation or aggregation of A\(\beta\), and such pharmaceutical composition can be used as an additive in food, drinks, chewing substance, patches, skin care products, etc. Another object of the present invention is to provide a pharmaceutical composition for preventing or treating A\(\beta\)-associated diseases or conditions.

Still another object of the present invention is to provide use of a pharmaceutical composition in the fabrication of a medicament for preventing or treating A\(\beta\)-associated diseases or conditions.

A pharmaceutical composition for preventing or treating A\(\beta\)-associated diseases or conditions provided in accordance with the present invention comprises acteoside and isoacteoside as potent components, wherein a weight ratio of the isoacteoside to the acteoside is 4:1 to 1:4.

Preferably, the weight ratio of the isoacteoside to the acteoside in the pharmaceutical composition is 4:1 to 2:3.

Preferably, the pharmaceutical composition is free of echinacoside.

Preferably, the pharmaceutical composition is able to inhibit formation, accumulation or aggregation of amyloid \(\beta\) peptides.

Preferably, the pharmaceutical composition is able to inhibit extracellular formation, accumulation or aggregation of amyloid \(\beta\) peptides.

Preferably, the pharmaceutical composition is able to inhibit neuronal damage or apoptosis caused by the amyloid \(\beta\) peptides, so as to retain, improve or restore learning and memory abilities.

Preferably, the A\(\beta\)-associated disease or condition is Alzheimer’s disease, mild cognitive impairment, Lewy body dementia, Down syndrome, Hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration, or Pick’s disease.

Preferably, the pharmaceutical composition is for treating Alzheimer’s disease.

Preferably, the pharmaceutical composition is for preventing an organism from suffering Alzheimer’s disease or for delaying an organism suffering Alzheimer’s disease.

Preferably, an effective dosage of the pharmaceutical composition to a person is equivalent to per day 0.2 mg to 4.0 mg of the potent components per kg of body weight.

Preferably, the pharmaceutical composition comprises a phenylethanoid glycoside preparation extracting from a plant as a source of the potent components, wherein the preparation comprises the isoacteoside to the acteoside as the major phenylethanoid glycosides, and the content of the isoacteoside is greater than that of the acteoside.

Preferably, the preparation comprises 12-32% of acteoside and 26-46% of the isoacteoside, based on the weight of the preparation.

Preferably, the plant is *Cistanche tubulosa* (Schenk.) Wight.

Preferably, the preparation is provided by a process comprising the following steps:
a) extracting fleshy stems of Cistanche tubulosa (Schenk.) Wight with a first polar solvent; 

b) introducing the resulting extract from step a) into a column which is packed with hydrophobic macro-porous polymeric beads, thereby enabling phenylethanoid glycosides to be adsorbed on the polymeric beads; 

c) eluting the column by use of a second polar solvent serving as a mobile phase, so that relatively less strongly adsorbed compounds are eluted from the column with most of phenylethanoid glycosides still being adsorbed on the polymeric beads; and 

d) eluting the column by use of a third polar solvent so as to obtain an eluate which contains phenylethanoid glycosides, wherein the first polar solvent is water, methanol, ethanol, a mixture of water and methanol, or a mixture of water and ethanol; the second polar solvent is water; and the third polar solvent is methanol, ethanol, a mixture of water and methanol, or a mixture of water and ethanol, and the third polar solvent is lower in polarity than the second polar solvent; 

e) concentrating the eluate which contains phenylethanoid glycosides, dissolving the concentrate in water, and contacting the aqueous solution with a macro-porous resin, so that the phenylethanoid glycosides are adsorbed on the macro-porous resin; and 

f) eluting the macro-porous resin with a fourth polar solvent and a fifth polar solvent in sequence, wherein the fifth polar solvent is lower in polarity than the fourth polar solvent, so that an eluate resulting from the fourth polar solvent elution contains only acteoside and isoacteoside, wherein the fourth polar solvent and the fifth polar solvent are a mixture of water and methanol or a mixture of water and ethanol.

[0022] Preferably, the fourth polar solvent is 25-35% ethanol aqueous solution and the fifth polar solvent is 35-45% ethanol aqueous solution.

[0023] To better understand the above and other objects, features and advantages of the present invention, the present invention will be described in detail below with examples presented with reference to the annexed drawings.

Brief Description of the Drawings

[0024] Fig. 1 shows the effects of drug A (acteoside), drug I (isoacteoside), C (control group, no drug), and pharmaceutical compositions having different ratios of A to I on extracellular A\(\beta\)_{1-40} accumulation.

Fig. 2 shows the effects of drug A (acteoside), drug I (isoacteoside), C (control group, no drug), and pharmaceutical compositions having different ratios of A to I on A\(\beta\)_{1-42} on A\(\beta\)_{1-42} oligomerization.

Best Modes of Embodying the Invention

[0025] Various diseases caused by A\(\beta\) have a common feature: formation of A\(\beta\) aggregates. These A\(\beta\) aggregates present in shapes such as fibrils or plaques, and deposit in systems, organs, tissues or body fluids of organisms, causing various diseases or conditions. It is therefore supposed that inhibition of A\(\beta\) formation, accumulation or aggregation can be used as an approach for effectively preventing or treating A\(\beta\)-associated diseases or conditions.

[0026] The term “prevent” used herein means avoiding or delaying occurrence of a disease or condition in organisms. The term “treat” used herein means slowing or stopping progress of a disease or condition, or making an individual return back to his improved or normal status.

[0027] The term “amyloid \(\beta\) peptide (A\(\beta\))-associated diseases or conditions” generally refers to those diseases or conditions that occur relating to formation, accumulation or aggregation of A\(\beta\), and particularly refers to the diseases or conditions that are caused by A\(\beta\). When abnormal formation, accumulation or aggregation is found in a certain proportion of individuals with certain diseases or conditions, the diseases or conditions can be considered as being associated with A\(\beta\). In addition, when A\(\beta\) aggregates somewhere that is close to occurrence of pathological features affected in certain diseases or conditions, the diseases or conditions can be also considered as being associated with A\(\beta\).

[0028] In the following examples test samples listed in Table 1 were used for carrying out the A\(\beta\) experiments, which were compared to a Vehicle control group which was not added with any test samples.

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<th>Symbol</th>
<th>Test sample</th>
<th>Concentration</th>
<th>Source</th>
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<tr>
<td>A</td>
<td>Acteoside</td>
<td>50 (\mu)g/ml</td>
<td>Sinphar Lab., purity 97%</td>
</tr>
<tr>
<td>I</td>
<td>Isoacteoside</td>
<td>50 (\mu)g/ml</td>
<td>Sinphar Lab., purity 97%</td>
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</table>
Example 1: Neuroblastoma cell culture

Wild-type human neuroblastoma cells (SH-SY5Y) were cultured in Eagle’s Minimum essential Medium (EMEM) / Ham’s F12 medium (1:1 mixture) (containing 10% FBS, 10 units/ml penicillin, 10 μg/ml Streptomycin). Wild-type mouse neuroblastoma Neuro-2a cells were cultured in minimum essential medium (MEM) (containing 10% FBS, 10 units/ml penicillin, 10 μg/ml Streptomycin).

Example 2: The effect of each test sample on extracellular Aβ₁-40 accumulation

The medium of the wild-type human neuroblastoma SH-SY5Y cells in Example 1 were switched into chemical defined medium (EMEM/F12 medium (Cat.No.12500-062), Hepes 5 mM, Glucose 0.6%, NaHCO₃ 3 mM, Glutamine 2.5 mM, Insulin 25 μg/ml, Transferin 100 μg/ml, Progestrone 20 nM, Putrescine 60 μM, Sodium selenite 30 nM, Heparin 2 μg/ml). Each well contained 1x10⁵ SH-SY5Y cells in 300 μl of culture medium. Thirty minutes later, each well was treated with the test samples given in Table 1 respectively at a total concentration of 50 μg/ml for 24 hours. After that, the level of Aβ₁-40 in the medium of each well was analyzed by Human Aβ₁-40 immunoassay kits (Catalog # KHB3482 Invitrogen).

Example 3: The effect of each test samples on Aβ₁-42 oligomerization

Dried Human Aβ₁-42 was taken out from the refrigerator and equilibrated to room temperature. Aβ₁-42 was dissolved in 1,1,1,3,3,3-Hexa-fluro-2-propanol (HFIP) to a concentration of 1mM, and was then placed at room temperature for one hour. The Aβ₁-42/HFIP solution was aliquoted by Hamilton syringe, and was then dried under a stream of nitrogen gas, followed by storing at a temperature of -20°C. Aβ₁-42 treated with HFIP was dissolved in PBS, and was vibration-incubated with treatment of each test sample at a concentration of 50 μg/ml and at 4°C for 24 hours to prepare Aβ₁-42 oligomers. The level of Aβ₁-42 oligomerization was analyzed by thioflavin T fluorescence (Ex=450 nm, Em=482 nm).
Example 4: A pharmaceutical composition contains substantially only acteoside and isoacteoside of the phenylethanoid glycosides. In one of the preferred embodiments of the present invention the fourth polar solvent and a fifth polar solvent in sequence, wherein the fifth polar solvent is lower in polarity than the fourth polar solvent.

The present invention provides a further purification process to obtain a pharmaceutical composition comprises acteoside and isoacteoside which are the only phenylethanoid glycosides contained therein by directly purifying the aforesaid phenylethanoid glycosides-containing preparation from *Cistanche tubulosa* (Schenk.) Wight. The further purification process comprises the steps of: e) purifying the aforesaid preparation from *Cistanche tubulosa* (Schenk.) Wight containing various phenylethanoid glycosides with a macro-porous resin; and f) eluting the macro-porous resin with a fourth polar solvent and a fifth polar solvent in sequence, wherein the fifth polar solvent is lower in polarity than the fourth polar solvent, so that an eluate resulting from the fifth polar solvent elution contains substantially only acteoside and isoacteoside of the phenylethanoid glycosides. In one of the preferred embodiments of the present invention the fourth polar solvent can be for example 25-35% ethanol aqueous solution and the fifth polar solvent can be for example 35-45% ethanol aqueous solution.

Preferably, the hydrophobic macro-porous polymeric beads are cross-linked polyaromatics, and more preferably cross-linked polystyrene or cross-linked copolymer of styrene and divinyl benzene, such as D-101 type or AB-8 type materials.

A pharmaceutical composition contains substantially only acteoside and isoacteoside of the phenylethanoid glycosides can be directly obtained by concentrating or drying the eluate resulting from the fifth polar solvent elution.

Example 4: A pharmaceutical composition contains substantially only acteoside and isoacteoside of the phenylethanoid glycosides.
preparation containing phenylethanoid glycosides and having a weight of 1107 g.

[0044] A high performance liquid chromatography (HPLC) was carried out under the following conditions: solvent A: acetonitrile containing 0.1% formic acid (CAN); solvent B: MQ-H2O containing 0.1% formic acid; column: Agilent Zorbax SB-C18 column of 2.1 x 150 mm, 5 μm; flow rate: 0.3 ml/min; and UV wavelength of 333 nm. The contents of echinacoside, acteoside and isoacteoside of the preparation containing phenylethanoid glycosides were measured, which were calculated as 33.6 wt%, 3.65 wt% and 6.05 wt%, respectively.

[0045] 200 g of the preparation containing phenylethanoid glycosides was dissolved in 800 g of water, and the resulting solution was introduced into a macro-porous resin to undergo purification, which was eluted with 30% ethanol aqueous solution and 40% ethanol aqueous solution in sequence. A thin layer chromatography was conducted with UV 365 nm to analyze each eluate, wherein the eluate collected from the 30% ethanol aqueous solution does not contain acteoside and isoacteoside, and the eluate collected from the 40% ethanol aqueous solution contain only acteoside and isoacteoside of phenylethanoid glycosides of 23.6 g. In this example, the acteoside in the eluate is 22.5 wt%, and the isoacteoside in the eluate is 36.4 wt%.

[0046] Although the present invention has been disclosed by several preferred embodiments described above, they are not for limiting the present invention. Various equivalent replacements and modifications made without departing from the spirit of the present invention by those skilled in the art should be still within the scope of the appended claims.

Claims

1. A pharmaceutical composition for use in preventing or treating amyloid β peptide-associated diseases or conditions comprising acteoside and isoacteoside as potent components, wherein a weight ratio of the isoacteoside to the acteoside is about 4:1 to about 1:4.

2. The pharmaceutical composition of claim 1, wherein the weight ratio of the isoacteoside to the acteoside is about 4:1 to about 2:3.

3. The pharmaceutical composition of claim 1, which is free of echinacoside.

4. The pharmaceutical composition claim 1, wherein the pharmaceutical composition inhibits formation, accumulation or aggregation of the amyloid β peptides.

5. The pharmaceutical composition claim 1, wherein the pharmaceutical composition inhibits extracellular formation, accumulation or aggregation of the amyloid β peptides.

6. The pharmaceutical composition of claim 1, wherein the pharmaceutical composition inhibits neuronal damage or apoptosis caused by the amyloid β peptides, so as to retain, improve or restore learning and memory abilities of said individual.

7. The pharmaceutical composition claim 1, wherein the amyloid β peptide-associated disease or condition is Alzheimer’s disease, mild cognitive impairment, Lewy body dementia, Down syndrome, Hereditary cerebral hemorrhage with amyloid (HCHWA) Dutch, Parkinsonism-dementia complex on Guam, Cerebral amyloid angiopathy, inclusion body myositis, frontotemporal dementia, age-related macular degeneration, or Pick’s disease.

8. The pharmaceutical composition of claim 1, wherein said pharmaceutical composition is for treating Alzheimer’s disease.

9. The pharmaceutical composition of claim 1, wherein said pharmaceutical composition is for preventing an organism from suffering Alzheimer’s disease or for delaying an organism suffering Alzheimer’s disease.

10. The pharmaceutical composition of claim 1, wherein an affective dosage of said pharmaceutical composition to human is equivalent to 0.2 mg - 4.0 mg of said potent components per kg of body weight per day.

11. The pharmaceutical composition of claim 3, which comprises a phenylethanoid glycoside preparation extracting from a plant as a source of the potent components, wherein the preparation comprises the isoacteoside and the acteoside as the major phenylethanoid glycosides, and the content of the isoacteoside is greater than that of the acteoside.
12. The pharmaceutical composition of claim 11, wherein the preparation comprises 12-32% of acteoside and 26-46% of the isoacteoside, based on the weight of the preparation.

13. The pharmaceutical composition of claim 12, wherein the plant is *Cistanche tubulosa* (Schenk.) Wight.

14. The pharmaceutical composition of claim 13, wherein the preparation is provided by a process comprising the following steps:

a) extracting fleshy stems of *Cistanche tubulosa* (Schenk.) Wight with a first polar solvent;
b) introducing the resulting extract from step a) into a column which is packed with hydrophobic macro-porous polymeric beads, thereby enabling phenylethanoid glycosides to be adsorbed on the polymeric beads;
c) eluting the column by use of a second polar solvent serving as a mobile phase, so that relatively less strongly adsorbed compounds are eluted from the column with most of phenylethanoid glycosides still being adsorbed on the polymeric beads; and

d) eluting the column by use of a third polar solvent so as to obtain an eluate which contains phenylethanoid glycosides, wherein the first polar solvent is water, methanol, ethanol, a mixture of water and methanol, or a mixture of water and ethanol; the second polar solvent is water; and the third polar solvent is methanol, ethanol, a mixture of water and methanol, or a mixture of water and ethanol, and the third polar solvent is lower in polarity than the second polar solvent;
e) concentrating the eluate which contains phenylethanoid glycosides, dissolving the concentrate in water, and contacting the aqueous solution with a macro-porous resin, so that the phenylethanoid glycosides are adsorbed on the macro-porous resin; and
f) eluting the macro-porous resin with a fourth polar solvent and a fifth polar solvent in sequence, wherein the fifth polar solvent is lower in polarity than the fourth polar solvent, so that an eluate resulting from the fourth polar solvent elution does not contain acteoside and isoacteoside, and an eluate resulting from the fourth polar solvent elution contain only acteoside and isoacteoside, wherein the fourth polar solvent and the fifth polar solvent are a mixture of water and methanol or a mixture of water and ethanol.

15. The pharmaceutical composition of claim 14, wherein the fourth polar solvent is 25-35% ethanol aqueous solution and the fifth polar solvent is 35-45% ethanol aqueous solution.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

See the extra sheet

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K, A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)


C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:

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“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search: 04 March 2013 (04.03.2013)

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Name and mailing address of the ISA

State Intellectual Property Office of the P. R. China

No. 6, Xitucheng Road, Jimensiao

Haidian District, Beijing 100088, China

Facsimile No. (86-10) 62019451

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Authorized officer

LIN, Guan

Telephone No. (86-10) 62411194
### INTERNATIONAL SEARCH REPORT
Information on patent family members

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INTERNATIONAL SEARCH REPORT

CLASSIFICATION OF SUBJECT MATTER:
A61K 31/7028 (2006.01) i
A61P 25/00 (2006.01) i
A61P 25/16 (2006.01) i
A61P 25/28 (2006.01) i

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Patent documents cited in the description

- US 7087252 B2 [0002] [0004]
- WO 2011157059 A1 [0003] [0004] [0005]
- US 7087252 B [0037]